only bring stability to the fibers, but also determine the specific orientation of individual peptides within these nanofibers.

2241-Pos Board B11
Second Virial Coefficients as Determined using Self Interaction Chromatography and Protein Aggregation in Solution
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One of the key parameters for describing protein self-interactions in solution is the second virial coefficient; B_{22}. Measurement of the second virial coefficient is important for describing the solution behaviour of proteins, especially as it relates to precipitation, aggregation and crystallisation phenomena. Due to its quick analysis time and use of small amounts of protein, Self Interaction Chromatography (SIC) is becoming an increasing popular approach. This paper includes a detailed description of the best experimental practise for running SIC experiments on aqueous protein solutions.
SIC data is presented here for a series of protein systems studied including lysozyme and lactoferrin, for a wide range of salts and their concentrations. The aggregation kinetics for these two proteins have also been obtained using Dynamic Light Scattering (DLS) for the same solution conditions. Remarkably, SIC confirmed the repulsive nature of B_{22} values of lactoferrin for all salts and concentrations tested. This data agreed completely with DLS data which confirmed the exception stability of lactoferrin to aggregation in solution. In contrast, aggregation behaviour of lysozyme was a strong function of salt species present as well as their concentrations. This aggregation behaviour correlated well with B_{22} values obtained by SIC. This work confirming the value of SIC as a useful technique for predicting the aggregation behavior of proteins in solution via B_{22} data.

2242-Pos Board B12
The Role of Interfaces in the Nucleation of Amyloid Nanotubes
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The nucleation and growth pathways of cross-ß peptide aggregates are widely studied but not fully understood. Using Amyloid-ß(16-22), the nucleating core of the Amyloid-ß protein, and a rhodamine 110 -labeled peptide (Rh1722) that co-assembles with Amyloid- ß(16-22) nanotubes without changing morphology, we investigate the role of interfaces in amyloid nucleation and self assembly. The high sensitivity of the Rhodamine 110 lifetime to its local environment provides a metric for structural heterogeneity, which is exploited in these studies using Fluorescence Lifetime Imaging Microscopy (FLIM). Specifically, we identify the importance of air-solvent and solvent-glass interfaces in the nucleation of cross-ß peptide nanostructures and apply FLIM to demonstrate that different interfaces can lead to distinct nucleation and self-assembly pathways and also to differences in the structure of assembled peptides.

2243-Pos Board B13
Single Molecule Studies of Interaction Between Alzheimer's Amyloid-β Peptides of different Lengths
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The oligomers formed by amyloid-β (Aβ) peptides are thought to play a causative role in Alzheimer's disease, possibly due to membrane permeabilization by small Aβ oligomers. Two primary variants of Aβ, of lengths 40 (Aβ40) and 42 (Aβ42) amino acids are produced at defined ratios in a normal individual. Changes in the ratio of Aβ40/Aβ42 have been shown to correlate strongly with the disease. Further, a mixture of the two forms of the peptide has been shown to exhibit different fibrillization kinetics in vitro and to elicit different extents of cellular toxicity. Thus, mixed Aβ40/Aβ42 oligomers can have different characteristics compared to oligomers formed by either one of the peptides. However, most research in the field focuses on one of the peptides at a time. Further, the Aβ oligomers are heterogenous, metastable and physiologically occur at low nanomolar concentrations, which makes it difficult for the use of conventional techniques to identify the toxic oligomers. In our work, we use single molecule methods to overcome these obstacles and study the formation and the evolution kinetics of mixed oligomers of Aβ40 and Aβ42 at different ratios in solution. We employ single molecule FRET and photobleaching of two different fluorophores attached to the N-terminal of Aβ40 and Aβ42 to determine the oligomer size and composition. Extending these studies to oligomer formation and binding kinetics of the two peptides on model membranes constructed from brain lipid extracts will provide a critical new understanding of how the stoichiometry of interaction of the two peptides both in solution and on the membrane surface affects the composition and permeabilizing potency of the resulting mixed oligomers.

2244-Pos Board B14
Structures and Dynamics in Amyloid-Beta Dimers: Effects of Zinc Binding and Chelation
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Alzheimer's disease (AD) is causally linked to the self-association of amyloid-β peptide (Aβ), a small protein of 39-43 amino acids. Historically, Aβ fibrils found in extracellular senile plaques were thought to be the pathogenic agents; however, recent evidence suggests that Aβ oligomers as small as dimers are more closely linked to Alzheimer's symptoms and progression. We have employed Förster resonance energy transfer (FRET) measurements to probe structures and dynamics in dimers of Aβ40. Results for single, surface-tethered dimers reveal two characteristic FRET efficiencies, perhaps indicative of two preferred dimer structures; these values are confirmed by fluorescence lifetime measurements in bulk solution. Here, we report comparative studies in the absence and presence of zinc, which is thought to promote Aβ oligomer formation at synapses. The effects of the zinc chelator clioquinol in reversing zinc-induced structural change will also be discussed.

2245-Pos Board B15
Comparison of Epitaxially and Solution-Grown Amyloid ß25-35 Fibrils
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Amyloid fibrils play a key role in a wide range of protein misfolding disorders. Amyloid ß peptides form self-associating fibrillar structures in Alzheimer's disease. The biologically active, toxic fragment of the full-length beta peptide, the Aß25-35 peptide is able to form an oriented network on mica by an epitaxial assembly mechanism. Whether the structure of the epitaxially grown fibrils is similar or identical to that of the fibrils assembled under equilibrium conditions is not known. To explore these differences, we investigated fibril structure and dynamics with atomic force microscopy, force spectroscopy (AFM) and Fourier transform infrared (FTIR) spectroscopy methods.
According to our AFM experiments the epitaxially-grown fibrils were significantly different from solution-grown fibrils in terms of their morphology and formation kinetics. The fibril height was 1-3 nm for the epitaxially-grown fibrils, whereas solution-grown fibrils were 7-40 nm thick. Unlike epitaxialy-grown fibrils, fibrils assembled in solution displayed a presumably helical structure. While fibril assembly in solution occurred on a time scale of hours to days, on mica surface fibrils appeared within a few minutes. The nanomechanical behavior of Aß25-35 fibrils was characterized by the appearance of force staircases which correspond to the force-driven unzipping and dissociation of protofilaments. Both types of fibrils showed similar plateau-like nanomechanical responses, however the plateau-force distribution was unimodal for epitaxially-grown fibrils and bimodal for solution-grown fibrils. The IR spectra contained an intense peak indicative of beta-sheet structure: 1630 cm-1 and 1623 cm-1 for epitaxially grown fibrils and for fibrils assembled in solution respectively. The shift in the amide I band towards smaller wavenumbers indicates a more compact structure. Thus, while both fibrils types display an underlying beta-sheet structure, they are slightly different: solution-grown fibrils are more compact with a pronounced axial periodicity.

2246-Pos Board B16
Cellular Internalization of Monomeric and Oligomeric Amyloid-Beta 42 Peptide
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Amyloid beta (Abeta) is the major component of extracellular plaques found in Alzheimer's disease (AD). Uptake of Abeta from extracellular to intracellular space, which is likely by endocytosis, appears to be an important process for understanding AD pathology (Friedrich et al., PNAS 2010; Hu et al., PNAS 2009). It may also be a promising target for treatment and prevention.
We aimed to study biophysical details of Abeta uptake, and to determine the uptake kinetics for different Abeta forms: monomers, oligomers, higher molecular mass aggregates of Abeta in and small fibrils. We used neuroblastoma (Sh-EP) cells as a model for neurons. Uptakes kinetic were followed by using the fluorescent labelled Abeta 42 that was monomerized and was purified by gel filtration. Abeta 42 peptide was added to the cell culture medium and the amount of intracellular aggregates was quantified using automated fluorescence microscopy.